African Journal of Biotechnology Vol. 11(72), pp. 13739-13745, 6 September, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.633
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

Chemical analysis, antimicrobial and antioxidant activities of eight extracts from *Schrankia leptocarpa* L.

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Accepted 23 May, 2012

***Schrankia leptocarpa DC*. (Mimosaceae) is an African medicinal plant, traditionally used by plants practitioners in Benin to cure malaria, diarrhea and stomach ache. There is little ethnobotanical and almost no chemical information available for this species. Eight extracts from the entire plant of *Schrankia leptocarpa* were prepared using diethyl ether, acetone, cyclohexane, methylene chloride, ethyl acetate, methanol, butanol and ethanol/water (20:80). Phytochemical analysis were performed and antibacterial was evaluated against four reference bacteria, *Staphylococcus aureus*, *Enteroccocus feacalis*, *Escherichia coli* and *Pseudomonas aeruginosa,* and four patient isolated bacteria using iodonitrotetrazolium microtest. The antioxidant properties were investigated using 2,2-diphenyl-2-picrylhydrazyl (DPPH) and superoxide anion radical scavenging activity. All extracts showed antimicrobial activity ranging from 0.078 to 5 mg/ml against one or more bacteria. The most potent extract was the diethyl ether extract with a minimum inhibitory concentration value of 75 µg/ml on *E. faecalis*. Phytochemical screening showed a wide variety of phytoconstituents such as steroids, terpenes, phenolic acid, flavonoids, tannins and alkaloids. The DPPH radical scavenging activity indicated that the radical scavenging activity ranged from 1.35 to 3.47 µg/ml. The superoxide anion radical scavenging showed inhibitory percentage ranging from 32.04 to 86%. Higher activity was observed with ethyl acetate extract. The results provide an evidence for the traditional use of *S. leptocarpa* for the treatment of infective diseases.**

Key words: *Schrankia leptocarpa*, antibacterial, antioxidant, 2,2-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion.

INTRODUCTION

Using plants for medicinal purposes is an important part of the culture and tradition in Africa. The use of natural products together with their therapeutic properties is ancient as human civilization. For a long time, mineral, plant and animal products were the main sources of drugs. The importance of medicinal plants and traditional health systems in solving the health care problems is gaining increasing attention (Kawsar et al., 2008). Medicinal plants produce enormous secondary metabolites with distinct biological properties that make them valuable as health products or as structural templates for drug discovery (Mariita et al., 2011). The world health organization (WHO) estimates that more than 80% of the world population is dependent (wholly or partially) on plant-based drugs (Orwa et al., 2008).

The increasing prevalence of antibiotic resistance is a major health problem worldwide (Eryilmaz et al., 2010). Then, the development of drug resistance in human pathogens against commonly used antibiotics has

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necessitated a search for new antimicrobial substances. Antimicrobial from plant origin have enormous therapeutic potential for various ailments (Kuete et al., 2008) and they are traditionally effective in the treatment of infectious diseases. There are several reports on antimicrobial activity of different herbal extracts (Subramanian et al., 2006; Balaji and Hriharan, 2007; Sampathkumar et al., 2008; Sri Rama and Nagamani, 2008). On the other hand, the studies on 'oxidative stress' and its adverse effects on human health have become a subject of considerable interest, it is well know that free radicals play a great role in several diseases. The damage they cause lead to development of diseases such as arteriosclerosis, hypentension, cancer, inflammation, renal failure, liver disease, etc (Wamtinga et al., 2006; Tiwari, 2004; Govindarajan et al., 2005). Many efforts have been made to discover new antimicrobial and antioxidant compounds from various kinds of sources such as micro-organisms, animals and plants (Tamokou et al., 2008; Potchoo et al., 2008; Asmah et al., 2006; Rached et al., 2010).

In Bénin, about 90% of people rely on traditional medicines and traditional health practitioners as the primary source of healthcare. *Schrankia leptocarpa* is one of the plants which are used traditionally to treat malaria, stomachache, weariness, eruptive fever and hypertension (Adjanohoun et al., 1989). Traditionally, this plant was used in combination with some other species such as *Croton lobatus*, *Thalia geniculata* and *Argemone mexicana* for the treatment of fever (malaria). It is also combined with *Ocimum graticimum* for the treatment of microbial infections especially by woman. The decoction of *Schrankia leptocarpa* was sometimes used to wash head in the treatment of headache. The purpose of the present study was to investigate the antibacterial and antioxidant activities of different extracts of *S. leptocarpa* a traditional medicinal shrub of Benin used alone or in combination with other plants to treat infectious diseases. The tested microorganisms included reference strains and fresh clinical strains isolated from pathologic products.

MATERIALS AND METHODS

The entire plant of *S. leptocarpa* was collected in September 2008 from Sèmè-Kpodji, department of Ouémé (Southern Benin). Botanical determination was performed by taxonomists from the Herbier National of Abomey-Calavi University, and voucher specimens (Houngnon 954b) were deposited at the same herbarium. The entire plant was air-dried, powdered (0.2 mm sieve) and subjected to extraction.

Preparation of extracts

The powdered entire plant (75 g) of *S. leptocarpa* was successively extracted with technical solvents of varying polarity: cyclohexane, diethyl ether, methylene chloride, ethyl acetate, methanol and butanol. Each extraction was done three times for 2 h with 250 ml.

In second part extraction, 20 g powdered entire plant were also exhaustively extracted four times with 150 ml of acetone and a mixture of distilled water and ethanol (v/v). The extracts were then filtered through Whatman filter paper (No. 1, Whatman international Ltd, Maidstone, England). The filtrate was evaporated to dryness at 40°C under vacuum to obtain each extract which were then stored at 4°C until testing.

Phytochemical analysis

Phytochemical study was used to identify the major compounds present using aluminium-backed TLC plates (Merck, Silica gel F_{254}), according to Wagner and Blat (2001) with slight modification in mobile phase. The TLC plates were developed using three solvent system: ethyl acetate/methanol/water (100:17:13) (EMW), chloroform/methanol (8:2) (CM) and chloroform (100%). Each TLC plate was sprayed with specific reagent. Flavonoids, alkaloids, terpene, saponins, steroids, tannin, anthraquinones and coumarin assay were performed.

Microorganisms

The reference bacterial strains used in this study were obtained from University of Strasbourg (France). Three Gram-negative and Gram-positive were used: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enteroccocus feacalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923. Four clinical isolated *E. coli* were also used for the assay.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the different extracts was evaluated by the microplate dilution method using tetrazolium violet to indicate growth of the bacteria (Eloff, 1998). Active cultures for assay were prepared by introducing one colony of bacteria in 5 ml of culture median. The suspensions were incubated under agitation at 37°C to achieve the concentration of 10^6 CFU/ml. Each extracts were reconstituted to 20 mg/ml with a mixture of water/acetone (v/v 1:1). The suspension obtained was homogenised and filtered using Millipore filter (0.45 µm). 100 µl of the extract were serial diluted in 96-well microplate. 100 µl of active culture (10^6 CFU/ml) where added to each well and the microplate was sealed and incubated at 37°C. After 18 h of incubation, 40 µl of 0.2 mg/ml solution of *p*-iodonitrotetrazolium violet (Sigma Aldrich) dissolved in distilled water were added to the microplate wells and incubated at 37°C for 1 h. The MIC values were then determined. Each test was performed in triplicate. Gentamicin was used as a positive control. It is usually not possible to compare activity of different extracts of fractions. The total activity allows making this comparison. Indeed, the total activity means the volume to which the extract or fraction obtained from 1 g of plant material can be diluted and still inhibits the growth of the tested microorganisms Eloff (2004).

DPPH radical scavenging activity

Qualitative 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was determined by means of the method previously used by Masoko and Eloff (2007). It is a rapid test in order to evaluate the antioxidant activity of each extract. In this method, the color change of 2,2-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma-aldrich) which is purple in color being reduced to diphenylpicryl hydrazine which is yellow in color was employed to determine if extracts contained compounds with antioxidant

capacity. The extracts were loaded onto a thin layer chromatography plate (Merck, Sil Gel F_{254}) which was then developed in ethyl acetate/methanol/water (100:17:13). The plate was then sprayed with 0.2% freshly prepared daily DPPH solution in methanol and the color change was observed after 5 to 15 min. Quantitative DPPH radical scavenging activity was evaluated as reported previously by Schmeda et al. (2003). Each experiment was repeated three times and quercetol was used as the positive control. The decoloring process (DP, violet to yellow) which shows the free radical decrease in the reaction medium was calculated as follow:

$$DP = [1-(A_S - A_B)] \times 100/A_C$$

The IC_{50} value which is the extract concentration causing 50% inhibition was calculated using the equation of linear regression of plots of concentrations of the tested samples. Quercetol and L-Ascorbic acid were used as positive control.

Hypoxanthine/xanthine oxidase assay

The superoxide anion radical scavenging activity was assessed according to the method of Parejo et al. (2003). Two concentrations (100 and 25 µg/ml) were tested for each extract. The mixture solution contained buffer phosphate (pH = 7.5; Sigma-Aldrich), 0.2 mM of hypoxanthine (Sigma Aldrich) and 1 mM of nitro-blue tetrazolium (Fluka Biochemika). 0.38 U/ml of xanthine oxidase (Sigma Aldrich) was added to mix and to initiate the reaction. The plate was sealed and incubated for 10 min in the dark at room temperature (20°C). After incubation, the plate was read at 560 nm using spectrophotometer Versa max microplate reader. The inhibitory percentage (%I) of extract was calculated according to the formula below. Each assay was performed at least in triplicate and quercetol was use as positive control.

$$\%I = \left[1- \frac{DO_{\text{Sample}} - DO_{\text{Blank sample}}}{DO_{\text{Control}} - DO_{\text{Blank control}}}\right] \times 100$$

RESULTS AND DISCUSSION

Chromatographic analysis

Phytochemical screening showed the presence of steroids, terpenes, phenolic acid, flavonoids, tannins and alkaloids in low concentration. No chemical data on the genus is available in the literature but in previous study, alkaloids were described in the fruit of *Schrankia uncinata* (Smolenski et al., 1973).

Minimum inhibitory concentration (MIC)

The antimicrobial activity was evaluated against four reference bacteria. The microplate dilution method using tetrazolium violet indicated that the eight tested extracts showed antimicrobial activity against various bacteria in different levels. The MIC is compiled in Table 2. For the reference tested bacteria, the results obtained here ranged from 78.1 to 5000 µg/ml. The most interesting antibacterial activity was obtained with acetone and diethylether extracts against *E. faecalis* with a MIC of 78.1 µg/ml. They were followed by cyclohexane, methylene chloride, ethyl acetate and butanol extracts (0.625 mg/ml). *E. faecalis* was the most susceptible bacteria to all extracts with MIC values between 0.078 and 2.5 mg/ml. *S. aureus* was second most sensible with MIC values of 0.625 to 2.5 mg/ml whereas *E. coli* was the most resistant bacteria. These results are comparable to those obtained by Eloff et al. (2005) on *Combretum woodii* with MIC values ranging from 0.04 to 2.5 mg/ml on *Enterococus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The antimicrobial activity obtained in this study could be attributed to tannins, flavonoids or phenolic acid present in the extract. Previous study showed that plants that contained tannins possess antimicrobial activity (Trease and Evans, 1978).

The MIC was also evaluated against four multiresistant *E. coli* named S1, S2, S3 and S4. The antibiogramms of these clinical strains were previously performed by Anago (2009). The Data are recorded in Tables 1 and 2. The results obtained indicate that diethylether, methylene chloride, ethyl acetate and methanol extracts exhibited antibacterial activity with MIC values ranging from 0.625 to 2.5 mg/ml. Previous pharmacological investigation indicated only antimalarial activity of methylene chloride and methanol extracts against both chloroquine-sensitive and chloroquine-resistant strains (Weniger et al., 2004). These results were consistent with the traditional use of *S. leptocarpa* as antimicrobial. In this study, many extracts were active against both gram positive (*S. aureus*) and Gram negative (*P. aeruginosa, E. faecalis, E. coli*) bacteria. Generally, gram-negative bacteria are more resistant compared to the Gram-positive (Cos et al., 2006). This could explain the results obtained for *E. coli* which is the most resistant bacteria.

The results of total activity compiled in Table 3 show that the total activity (TA) vary according to extracts but also to the bacterial strain. The strongest activities were obtained with diethylether and acetone extracts with TA values of 247.5 and 307.3 ml against *E. faecalis*. The best total activity on all tested bacteria was obtained with the methanol extract (37.33 to 74.66 ml) whereas the least activity was obtained with cyclohexane (<1.36 to 10.88 ml). On the other hand, the TA was less strong on multi-resistant isolated bacteria than reference strains (<1.36 to 74.66 ml). These results confirmed the multi-resistant form and antibiogramms of the isolated bacteria.

DPPH radical scavenging activity

Qualitative DPPH free radical scavenging activity

The chromatogram (Figure 1) shows the results of qualitative DPPH free radical scavenging test. The

Table 1. Antibiogramms of clinical isolated bacteria.

Clinical *E. coli*	Sensible	Resistant
S1	Amoxicilin + Clavulanic acid, Ceftriaxon, Aztreonam, Cefotoxin, Ciprofloxacin, Ofloxacin, Nalidixic acid, Cefalotin, Cefazolin, Gentamicin	Amoxicilin, Doxycyclin, Nalidixic acid
S2	Amoxicilin + Clavulanic acid, imipeneme, Ciprofloxacin, Ofloxacin, Gentamicin	Amoxicilin, Ceftriaxon, Aztreonam, Cefotoxin, Doxycycline, cefalotine, cefazoline
S3	Ceftriaxone, Aztreonam, Cefotoxine, imipeneme, Ciprofloxacine, Ofloxacine, Nalidixic acid, Cefalotin, Cefazolin, Gentamicin.	Amoxicilin, Doxycyclin, Netilmycin
S4	Amoxicilin + clavulanic acid, Ceftriaxon, Cefotoxin, Imipeneme, Ciprofloxacin, Ofloxacin, nalidixic acid, netilmycin, Gentamicin	Cefalotin, Cefazolin, Amoxicilin, aztreonam, Doxycyclin

Table 2. Minimum Inhibitory concentrations (MIC) of extracts.

Extract	Reference strain				Clinical strain			
	E. coli	*S. aureus*	*P. aeruginosa*	*E. faecalis*	S1	S2	S3	S4
DEE	0.625	0.625	1.25	0.0781	0.625	0.625	1.25	0.625
CH	5	1.25	2.5	0.625	>5	>5	5	1.25
DM	2.5	1.25	1.25	0.625	0.625	0.625	2.5	0.625
ACE	2.5	2.5	1.25	0.625	0.625	0.625	2.5	0.625
MeOH	2.5	2.5	2.5	2.5	1.25	1.25	2.5	1.25
EtOH/H_2O	>5	1.25	>5	2,5	-	-	-	-
BuOH	2.5	0.625	2.5	0.625	>5	2.5	2.5	2.5
Acetone	5	0.625	1.25	0.0781	-	-	-	-
Gentamicine	0.06	0.06	0.08	0.02	-	-	-	-

DEE, Diethyl ether; CH, cyclohexane; DM, methylene chloride; ACE, ethyl acetate; MeOH, methanol; EtOH/H_20, ethanol/water; BuOH, butanol.

Table 3. Total activity of extracts.

Extract	Reference strain				Clinical strain			
	E. coli	*S. aureus*	*P. aeruginosa*	*E. faecalis*	S1	S2	S3	S4
DEE	30.93	30.93	15.46	**247.5**	30.9	30.93	15.46	30.93
CH	1.36	5.44	2.72	10.88	<1.36	<1.36	1.36	5.44
DM	1.87	3.74	3.74	7.47	7.47	7.47	1.87	7.47
ACE	3.15	3.15	6.3	12.59	12.6	12.59	3.15	12.59
MeOH	37.33	37.33	37.33	37.33	74.7	74.66	37.33	74.66
Et/H_20	<14.4	57.6	<14.4	28.8	-	-	-	-
BuOH	17.07	68.27	17.07	68.27	17.1	17.07	17.07	17.07
Acetone	4.8	38.4	19.2	**307.3**	-	-	-	-

DEE, Diethyl ether; CH, cyclohexane; DM, methylene chloride; ACE, ethyl acetate; MeOH, methanol; EtOH/H_20, ethanol/water; BuOH, butanol.

assay showed that extracts contained a number of secondary metabolites with antioxidant activity. Except cyclohexane and diethylether extracts, the other extracts showed antioxidant activity. This activity could be attributed to polar constituents. The ethyl acetate appears as the best solvent of extraction of antioxidant compounds of *S. leptocarpa*. The quantitative radical scavenging activity of extracts is showed in Table 4. This study was based on the measurement of the inhibitory effect of extracts tested at different concentrations. The results reveal that all of them having various degrees of antioxidant properties, with IC_{50} values ranged from 4.31 to 1.29 µg/ml. Ethyl acetate extract was significantly the most active extracts with an IC_{50} value of 1.29 µg/ml and

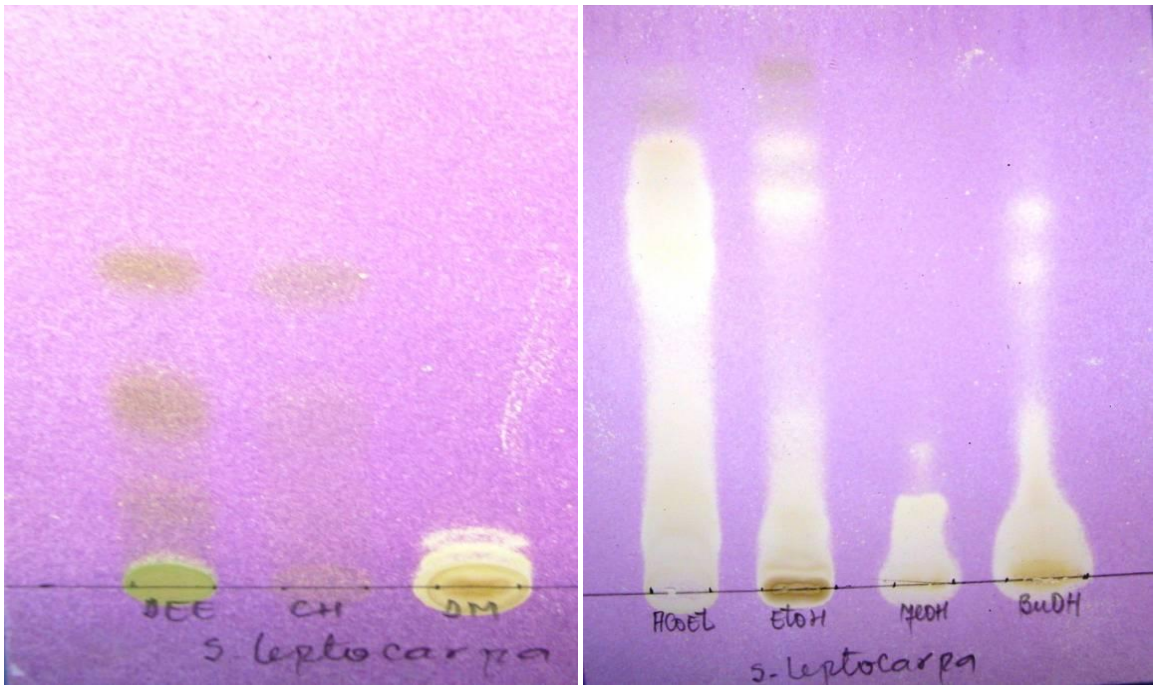

Figure 1. Qualitative DPPH radical scavenging activity. DEE, Diethyl ether; CH, cyclohexane; DM, methylene chloride; ACE, ethyl acetate; MeOH, methanol; EtOH/H_20, ethanol/water; BuOH, butanol.

Table 4. Quantitative DPPH radical scavenging activity of extracts.

Extract	DPPH radical scavenging activity	
	IC_{50} (µg/ml)	R2
Cyclohexane	4.31	0.88
Diethylether	1.58	0.52
Methylene chloride	3.18	0.89
Ethyl acetate	1.29	0.99
Methanol	1.35	0.82
Butanol	1.52	0.94
H_20/EtOH	1.85	0.90
L-Acid ascorbic	1.1	0.90
Quercetol	0.094	0.95

good correlation index (R^2 = 0.99). The hydroethanol and butanol extracts also exhibited good activity (1.85 and 1.52 µg/ml, respectively). The free radical scavenging activity is due in part to the presence of flavonoids, tannins and phenolic acid. Numerous authors reported that these chemical phytoconstituents could contribute to the antioxidant activity of extracts (Galvez et al., 2005; Pieters and Vlietinck, 2005; Potchoo et al., 2008).

Hypoxanthine/xanthine oxidase assay

Superoxides are produced from molecular oxygen due to oxidative enzymes (Sainani et al., 1997) of the body as well as via non-enzymatic reaction such as autooxidation by catecholamines (Hemmani and Parihar, 1998).

In the present study, superoxide radical reduced NBT to a blue colored formazan that is measured at 560 nm (Khanam et al., 2004). The decrease of absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture. The superoxide anion radical scavenging activities of extracts are showed in Figure 2. At 100 µg/ml, ethyl acetate extract showed interesting activity by inhibiting 86% of superoxide anion radical whereas ethanol and butanol extracts inhibited 55.87 and 32.03%, respectively. At 25 µg/ml, ethyl acetate extract was also the most active with an inhibitory percentage of 46%. Butanol and ethanol extracts showed slight activity

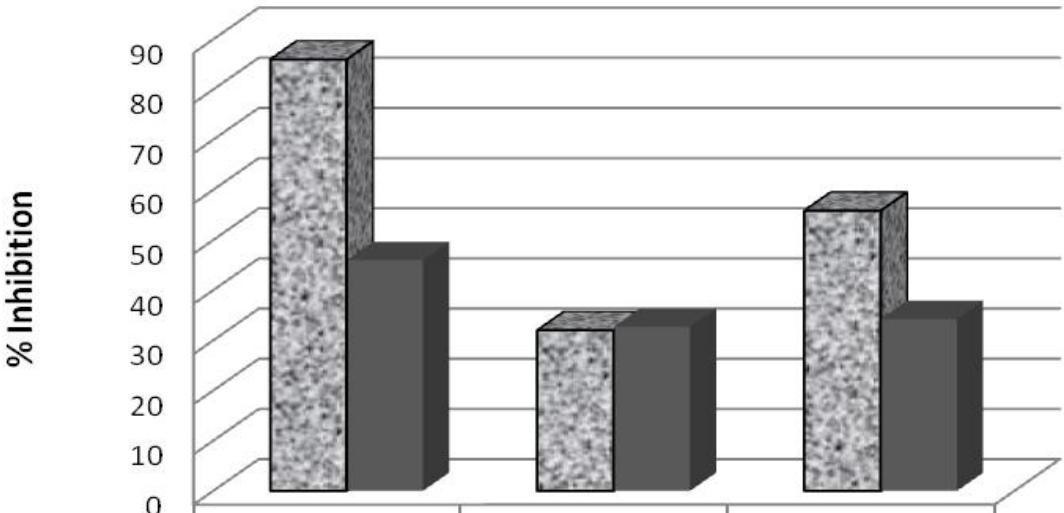

Figure 2. Inhibitory percentage of superoxide anion radical scavenging. SL, *Schrankia leptocarpa*; ACE, ethyle acetate; BuOH, butanol; H_2O/EtOH, water/ethanol.

(32.74 and 34.27%). These results are in accordance with those obtained in DPPH free radical scavenging activity.

Conclusion

Plants have played a significant role in human health. They have served as ingredients for medicines. Numerous *in vitro* studies have shown that some of the phytoconstituents are potent biological activities. Three main axes guided this study: determination of the MIC of extracts against reference strains and patient isolated bacteria, the phytochemical study of *S. leptocarpa* and finally the evaluation of the antioxidant activity. The results obtained here show that *S. leptocarpa* have a good antibacterial and antioxidant activities. These results confirm the use of this plant in traditional medicine for the treatment of microbial infections. Further investigations are in progress in our laboratory to identify the active principles involved in this antioxidant and antimicrobial activities.

ACKNOWLEDGEMENTS

The authors wish to thank the plants sellers from south Benin for their willingness to share with us their knowledge about the plants. The authors also thank International Foundation for Science (IFS) and Organization of Prohibition of Chemical Weapons (OPCW) for the financial support.

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